

# Biodiversity of Arbuscular Mycorrhizal (AM) fungi in mangroves of Goa in West India

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**Abstract:** Seventeen mangrove species of eight families at seven riverine and fringe habitats in Goa West India were surveyed for Arbuscular Mycorrhizal (AM) fungal diversity. Sixteen species were found to be mycorrhizal and one species showed no AM fungal colonization. AM root colonization was recorded at all seven sites and ranged from 6%–77%. Maximum root colonization was recorded in *Excoecaria agallocha* (77%) and minimum colonization in *Avicennia marina* (6%). *Paris*-type colonization was predominant at all sites. Auxiliary cells were recorded in roots of *Acanthus ilicifolius*, *Ceriops tagal* and *Sonneretia alba*. AM fungal root colonization and spore density varied by plant species and site. Site average spore density ranged from 1.84 spores·g<sup>-1</sup> to 0.54 spores·g<sup>-1</sup> of soil. In total, 28 AM fungal species of five genera, viz. *Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora* and *Entrophospora*, were recovered. *Glomus* was the dominant genus, three species of which were sporocarpic forms. Maximum site species richness (SR) ranged from 16 to 5. Species richness was maximum in *A. ilicifolius* where seven species of three genera were recovered. Based on relative abundance (RA) and isolation frequency (IF), two common species, viz. *G. intraradices* and *A. laevis*, were recovered from all seven sites.

**Keywords:** *Glomus intraradices*; root colonization; *Paris* type; spore density; rhizosphere

## Introduction

Mangroves are facultative halophytes, characterized by regular tidal inundation and fluctuating salinity (Gopal and Chauhan 2006). Mangrove plant species are highly adapted to coastal

environments, both morphologically and physiologically, and thrive in intertidal zones of tropical and sub-tropical regions (Ball 1996; Naidoo et al. 2002). They exhibit exposed breathing roots, extensive support roots and buttresses, salt-excreting leaves and viviparous water-dispersed propagules. These adaptations vary among taxa and with physico-chemical variations of habitat (Duke 1990). Distribution is governed by topography, tidal height, substratum and salinity. Mangroves display extreme variations in species composition, forest structure and growth rate. Mangrove forests can vary from a narrow fringe along the banks of an estuary to dense stands covering many square kilometers. Total mangrove area in India is 6,740 km<sup>2</sup>, of which 80% is found along the east coast and 20% on the west coast. Deltaic environments on Indian east coast support extensive mangrove forests due to intertidal slope and the heavy impact of siltation. The western coastline has narrow intertidal belts which support only fringe mangroves. All estuaries in Goa are classified as microtidal because the tidal range is below two meters (Ahmad 1972). Mangroves have become the center of many conservation and environmental issues because of loss of beneficial effects on the coastal environment. Anthropogenic pressure is constantly increasing and immediate protection and conservation of the ecosystem is necessary. Reforestation of mangrove is a promising solution to restoration.

Ecological functions attributable to Arbuscular Mycorrhizal (AM) fungi include helping to increase plant tolerance of adverse soil conditions, influencing response to severe climatic conditions and increasing plant productivity in natural plant communities (Brundrett and Kendrick 1996). AM-enhanced availability of nutrients is described as a primary factor affecting abundance and composition of plant species communities (Klironomos 2003). The major nutrients phosphorus (P) and nitrogen (N) are deficient in mangrove ecosystems (Carr and Chambers 1998) and likely to limit the growth of mangrove plant species.

Microorganisms such as phosphate-solubilizing, N-fixing and AM fungi are known to interact in rhizosphere soils and can solubilise the bound P into available form. AM fungal hyphae aid in transport of nutrients by extending beyond the depletion zone (Cui and Cladwell 1996). AM fungi play significant roles in

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physiological processes such as water use efficiency (Ruiz-Lozano et al. 1996), modifying the structure and function of plant communities, and are useful indicators of ecosystem change (Miller and Bever 1999). Burke et al. (2003) demonstrated that inoculation with AM fungi improves growth of plants under salinity stress. Previous studies have shown that these fungi are either absent (Mohankumar and Mahadevan 1986), rare (Kothamasi et al. 2006) or ubiquitous (Sengupta and Chaudhuri 2002; Kumar and Ghose 2008) in mangrove ecosystems. In India, most studies of mangroves and AM fungi were carried out along the east coast while studies on the west coast are scarce. In addition to their use in afforestation, established AM fungal plant species might serve as important sources of inocula for initially non-mycorrhizal conspecifics, thereby affecting their regeneration and contributing to overcome the patchy distribution of species within communities (Koide et al. 2000). Hence, it is important to study the diversity of AM fungal species and identify their potential for use in afforestation using native species in mangrove habitats. The aim of our research was to quantify AM fungal diversity at selected mangrove sites in Goa and to identify the dominant AM fungal species found therein.

## Materials and methods

### Study sites and sample collection

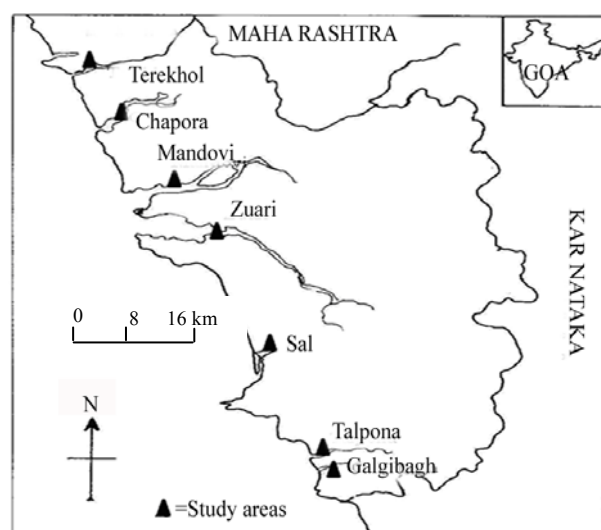
Goa is located on the west coast of India. The state has 12,000 ha of estuaries, 2,000 ha of which are occupied by mangrove forest. We selected seven sites covering all the major estuaries of Goa (Fig. 1; Table 1) for this study. Tropical weather at the sites is warm and humid, and soils are marshy. The mean temperature range is 22–35 °C and average annual rainfall is 2,500 mm. In total, 17 mangrove species of eight families, viz. Acanthaceae, Rhizophoraceae, Euphorbiaceae, Myrsinaceae, Salvadoraceae, Sonneratiaceae, Ceratopteridaceae and Fabaceae, were investigated (Table 1). Of these, 14 were true mangrove species and 3 were mangrove associates, identified following Rao (1985). Root and rhizosphere soil samples were randomly collected from June 2007 to September 2009 from all study sites. During collection, care was taken to ensure that the collected roots belonged to the same plant. Fine roots of mature trees were traced by digging, and removed with adhering soil. The samples were collected in polyethylene bags and brought to the laboratory. The roots were separated from adhering soil, washed gently under tap water and fixed in FAA (formalin-acetic acid-alcohol) for estimation of AM colonization. Rhizosphere soil of individual plants was air dried at room temperature, sieved (mesh size 720 µm) and divided into two parts, one for isolation, enumeration and identification of AM spores, the other as inoculum for trap culture.

### Soil analyses

Subsamples from each of the study sites were collected in polyethylene bags from depths of 0–25 cm and air-dried in the laboratory before passing through a 2-mm sieve and mixed thoroughly to obtain composite samples. Soil pH was measured in soil:water (1:2) suspension using a pH meter (LI 120 Elico, In-

dia). Electrical Conductivity (EC) was measured at room temperature in a 1:5 soil suspension using a Conductivity Meter (CM-180 Elico, India). Standard soil analysis techniques, viz. Walkley and Black (1934) rapid titration method and Bray and Kurtz method (1945) were employed for determination of organic carbon and available P, respectively.

Available potassium was estimated by the ammonium acetate method (Hanway and Heidel 1952) using a flame photometer (Systronic 3292). Available zinc, copper, manganese and iron were quantified by the DTPA-CaCl<sub>2</sub>-TEA method (Lindsay and Norvell 1978) using an Atomic Absorption Spectrophotometer (AAS 4139).



**Fig. 1** Map of Goa showing mangrove study sites

### Estimation of AM fungal root colonization

Fixed roots were placed in 2% KOH, heated at 90 °C, acidified with 1% HCl and stained with trypan blue (Koske and Gemma 1989). The stained roots were examined on a compound microscope (100X–1000X) for AM fungal structures and percent root colonization was estimated using the slide method (Giovannetti and Mosse 1980). A segment was considered mycorrhizal when it showed the presence of hypha, arbuscule or vesicle.

### Trap culture, isolation and taxonomic identification of AM fungal spores

To propagate spores for identification, trap cultures were prepared in pots by using field soil and sterile sand (1:1). *Solenostemon scutellarioides* (L.) was used as the catch plant, maintained in a polyhouse at 27 °C. All cultures were provided 16h day/8h night photoperiod for a period of six months. The pots were watered as and when required and Hoagland's solution (-P) was added fortnightly to the pots.

Spores used for identification were obtained from both rhizosphere soil samples and trap cultures and were isolated using wet sieving and decanting techniques (Gerdemann and Nicolson 1963). Intact and crushed spores mounted in Poly vi-

nyl-lacto Glycerol (PVLG), (Koske and Tessier 1983) were examined under an Olympus BX 41 compound microscope. Identifi-

cation was based on spore morphology and sub-cellular characters (Schenck and Perez 1990).

**Table 1. Geographic location and physico-chemical analysis of the study sites**

Sites	pH	EC (d-Sm <sup>-1</sup> )	OC (%)	P (g·kg <sup>-1</sup> )	K (g·kg <sup>-1</sup> )	N (g·kg <sup>-1</sup> )	Zn (g·kg <sup>-1</sup> )	Mn (g·kg <sup>-1</sup> )	Cu (g·kg <sup>-1</sup> )	Fe (g·kg <sup>-1</sup> )
Terekhol (28 km) 15°72'28"N & 73°72'99" E	6.7	3.30	5.45	0.13	7.34	0.61	0.011	0.043	0.052	0.143
Chapora (31 km) 15°63' 98"N & 73°73'61" E	6.0	3.86	0.92	0.44	67.43	0.69	0.014	0.038	0.062	0.234
Mandovi (81 km) 15°48' 64"N & 73°86'52" E	5.5	4.59	1.30	0.24	62.72	0.29	0.022	0.011	0.042	0.425
Zuari (67 km) 15°32' 56"N & 73°89'71" E	5.9	8.49	2.0	traces	85.12	0.81	0.016	0.032	0.040	0.563
Sal (35 km) 15°15' 52"N & 73°95'30"E	6.0	5.25	1.80	0.40	56.04	0.62	0.013	0.015	0.022	0.192
Talpona (9 km) 14°98' 75"N & 74°06'15" E	6.4	2.40	4.20	0.41	65.37	1.21	0.025	0.044	0.037	0.242
Galgibagh (16 km) 14°95' 83"N & 74°04'95" E	6.4	2.19	3.35	0.32	64.99	0.21	0.032	0.072	0.015	0.283

## Diversity studies and Statistical analyses

Diversity studies were conducted for each site separately by calculating Simpson's Diversity index  $1-D$  (Simpson 1949),  $D=1-\sum (P_i)^2$ , where,  $P_i = n_i/N$ , ( $n_i$ ) the relative abundance of the species, is calculated as the proportion of individuals of a given species ( $n_i$ ) to the total number of individuals in a community ( $N$ ). Simpson's reciprocal index was calculated using the following formulae,  $1/D$ . Shannon diversity index ( $H$ ) is commonly used to characterize species diversity in a community, which accounts for both abundance and evenness of the species present,  $H = -\sum (P_i \ln (P_i))$  (Shannon and Weaver 1949). Species evenness ( $E$ ), which indicates the distribution of individuals within species was calculated by using the formula:  $\Sigma (H) = H'/H'_{\max}$ , where  $H'_{\max} = \ln S$ ,  $S$  = total number of species in the community (richness). Isolation Frequency (%) of each species was calculated as  $(s_i/S) 100$ , where  $s_i$  is the number of soil samples containing spores of the  $i^{\text{th}}$  species and  $S$  is the total number of soil samples examined.

Pearson's correlation coefficient was calculated to assess the relationships between root colonization and spore density, isolation frequency and relative abundance, and spore density and species richness at each site, using WASP software (Web Based Agricultural package) 2.0 ( $p \leq 0.05$ ). By using WASP software, relative abundance of AM fungal species common to all sites was correlated with soil pH, Phosphorus, and EC ( $p \leq 0.05$ ).

## Results

### Soil analyses and AM colonization

Results of the soil physico-chemical analyses are shown in Table 1. Soils were acidic (pH range of 5.5–6.7). EC ranged from 2.19 to 8.49 d-Sm<sup>-1</sup>. Soils at all study sites were deficient in available P. With the exception of *Salvadora persica*, all investigated plant species were mycorrhizal. Colonization was characterized by presence of hyphae, arbuscules and vesicles. AM fungal colonization varied by species and the situation of their occurrence. Percent colonization was maximal for *E. agallocha* (77%) and minimal for *A. marina* (6%) (Appendix 1). Both *Arum*- and

*Paris*-type morphologies were observed, the latter type was dominant, observed in 74% of the plant species. *A. ilicifolius* was common to all study sites and showed variation in AM colonization (Appendix 1).

### Spore density and species richness

Spore density varied in the rhizosphere soils of selected plant species, with maximum spore density for *A. ilicifolius* (3.24 spores·g<sup>-1</sup> soil) and minimum for *D. heterophylla* (0.08 spores·g<sup>-1</sup> soil), (Appendix 1). Maximum spore density (1.84 spores·g<sup>-1</sup> soil) was recorded at the Sal site and minimum spore density was recorded at Talpona (0.54 spores·g<sup>-1</sup> soil). *G. intraradices* and *A. laevis* were recovered at all sites (Appendix 1). *A. ilicifolius* supported maximum species richness with seven AM fungal species of four genera. Zuari site showed highest species richness (16) with lowest richness (5) at Talpona (Table 2).

### Diversity and distribution of AM fungi

Twenty eight AM fungal species of five genera, viz. *Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora* and *Entrophospora*, were recovered from rhizosphere soils of all study sites (Fig. 2, a-i). *Glomus* (16 species) was the dominant genus followed by *Acaulospora* (6 species), *Scutellospora* (4 species), *Gigaspora* (1 species) and *Entrophospora* (1 species) (Table 3).

RA and IF were greatest for *Glomus* and least for *Entrophospora*. Within AM species, highest IF was recorded for *G. intraradices* followed by *G. geosporum*, *A. scrobiculata*, *A. laevis* and *A. bireticulata*, and lowest in *G. clarum* followed by *G. nanolumen*, *G. rubiforme*, *A. foveata*, *S. weresubiae* and *E. infrequens*. Maximum RA was recorded for *G. intraradices* followed by *A. scrobiculata* and *A. laevis* while minimum RA was recorded for *G. albida* followed by *S. calospora* and *A. delicata* (Table 3).

*G. mosseae* (25.4% IF, 2.02% RA) and *A. scrobiculata* (58.3% IF, 2.98% RA) at Mandovi and *G. aggregatum* (28.5% IF, 3.09% RA) at Sal had low relative abundance but were widely distributed (high IF). In contrast, *G. fasciculatum* (8.3% IF, 17.1% RA) at Mandovi and *G. clarum* (7.1% IF, 25.9% RA) at Zuari showed lower IF but were dominant in sporulation in comparison to other species. *G. intraradices* and *A. laevis* were recovered from all

seven sites. However, RA of both species showed no significant correlation with soil pH, P or EC ( $p \leq 0.05$ ).

Species evenness was highest at Talpona and lowest at Zuari. Shannon-Wiener diversity ( $H'$ ) was highest at Talpona and lowest at Zuari. Simpson's dominance index ( $D$ ) ranged from 0.97 to 0.99 (Table 2). Spore density was significantly correlated with

species richness at six sites while at Chapora ( $r=0.720$ ;  $p \leq 0.05$ ) there was no significant correlation (Table 4). Six of the seven sites, showed significant positive correlation between RA and IF, while Talpona ( $r=0.834$ ;  $p \leq 0.05$ ) showed no significant correlation. There was no significant correlation between spore density and root colonization at any site (Table 4).

**Table 2. Diversity of AMF communities at selected study sites**

Ecological parameters	Terekhol	Chapora	Mandovi	Zuari	Sal	Talpona	Galgibagh
Shannon- Wiener index of diversity (H)	0.66	0.60	0.44	0.40	0.44	0.78	0.55
Simpson's index of dominance (D)	0.98	0.98	0.999	0.999	0.999	0.97	0.99
AMF species evenness (E)	0.34	0.31	0.22	0.21	0.22	0.41	0.28
AMF species richness (SR)	7	6	12	16	8	5	6

**Table 3. Relative abundance (RA) and isolation frequency (IF) of AM fungi at by study site**

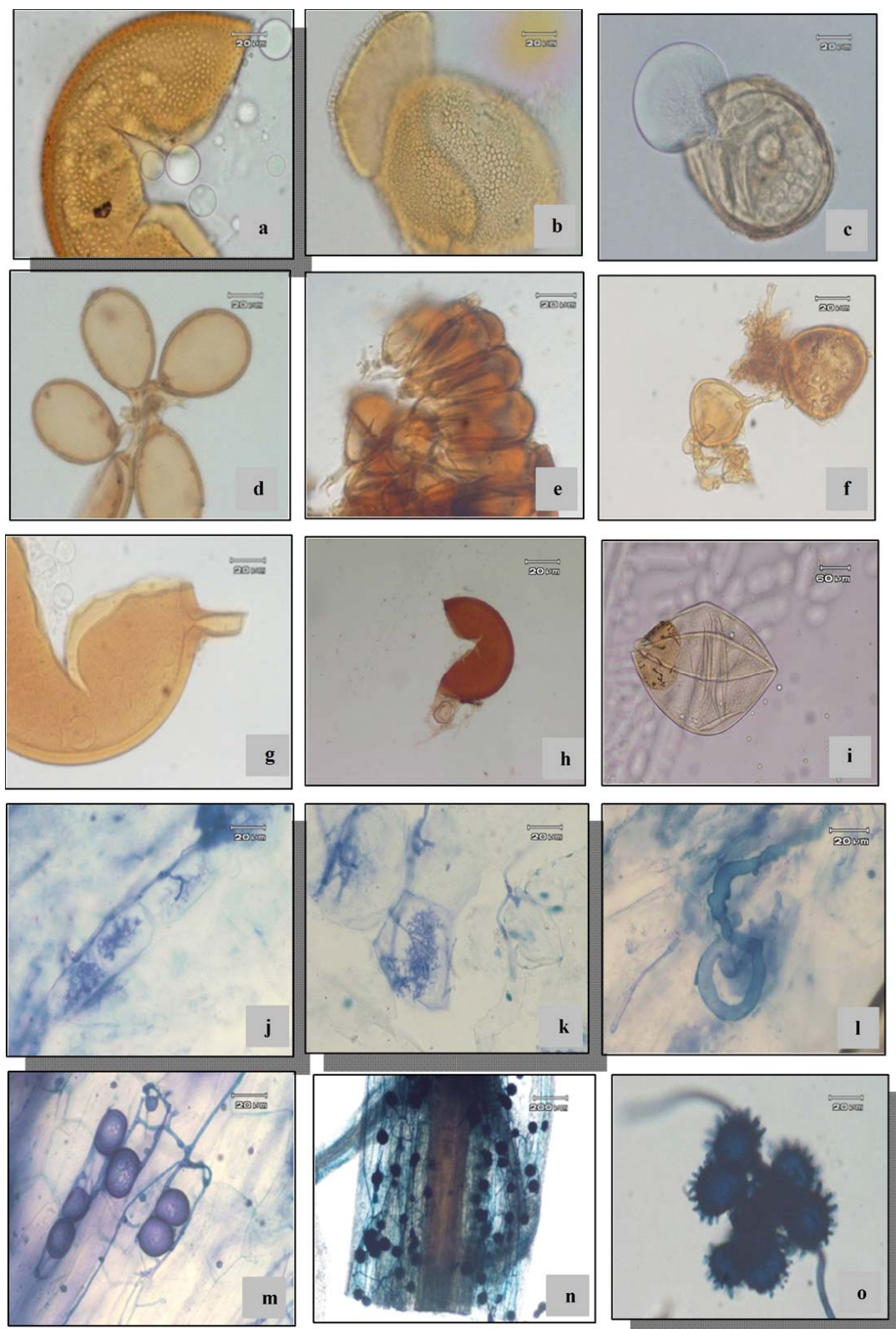
Sp. no.	AM fungal species	Terekhol		Chapora		Mandovi		Zuari		Sal		Talpona		Galgibagh	
		IF	RA	IF	RA	IF	RA	IF	RA	IF	RA	IF	RA	IF	RA
1	<i>Glomus intraradices</i> Schenck & Smith	60.0	49.4	42.8	48.6	33.3	14.7	71.4	25.0	80.0	51.9	50.0	29.5	60.0	43.9
2	<i>Glomus clarum</i> Nicolson & Smith	-	-	-	-	-	-	7.1	25.9	-	-	33.3	24.61	40.0	22.70
3	<i>Glomus multicaulis</i> Gerdemann & Bakshi	40.0	10.4	-	-	-	-	31.4	3.30	-	-	-	-	-	-
4	<i>Glomus aggregatum</i> Schenck & Smith	-	-	-	-	16.6	1.38	-	-	28.5	3.09	-	-	-	-
5	<i>Glomus mosseae</i> (Nicol. & Gerd.) Gerd. & Trappe	-	-	-	-	25.4	2.02	14.2	0.89	-	-	-	-	-	-
6	<i>Glomus fasciculatum</i> (Thaxter) Almeida & Schenck	-	-	28.5	8.45	8.3	17.1	-	-	-	-	-	-	20.0	20.90
7	<i>Glomus geosporum</i> (Nicol. & Gerd.) Walker	-	-	-	-	-	-	36.2	11.27	72.7	14.80	-	-	-	-
8	<i>Glomus hyderabadensis</i> Swarupa, Kunwar, Prasad, & Manohar	-	-	-	-	-	-	21.4	1.52	-	-	-	-	-	-
9	<i>Glomus formosanum</i> Wu & Chen	-	-	-	-	8.3	1.70	-	-	-	-	-	-	20.0	2.88
10	<i>Glomus nanolumen</i> Koske & Gemma	-	-	-	-	-	-	7.1	5.99	-	-	-	-	-	-
11	<i>Glomus constrictum</i> Trappe	-	-	-	-	-	-	14.2	3.84	-	-	-	-	-	-
12	<i>Glomus taiwanense</i> Wu & Chen	20.0	7.93	-	-	-	-	21.4	2.14	-	-	-	-	-	-
13	<i>Glomus rubiforme</i> Gerdemann & Trappe	-	-	-	-	8.3	12.5	7.1	3.13	60.0	7.28	-	-	-	-
14	<i>Glomus etunicatum</i> Becker & Gerd.	40.0	9.52	-	-	33.3	19.6	-	-	-	-	-	-	-	-
15	<i>Glomus maculosum</i> Miller & Walker	20.0	0.90	-	-	-	-	-	-	-	-	-	-	-	-
16	<i>Acaulospora foveata</i> Trappe & Janos	-	-	-	-	-	-	7.1	1.07	-	-	-	-	-	-
17	<i>Acaulospora bireticulata</i> Rothwell & Trappe	-	-	-	-	16.6	18.5	42.8	10.91	-	-	-	-	-	-
18	<i>Acaulospora delicata</i> Walker, Pfeffer & Bloss	-	-	-	-	8.3	0.74	-	-	-	-	-	-	-	-
19	<i>Acaulospora laevis</i> Gerdemann & Trappe	20.0	18.82	42.8	26.39	41.7	27.2	21.4	1.25	28.5	3.15	50.0	8.0	20.0	8.46
20	<i>Acaulospora mellea</i> Spain & Schenck	-	-	-	-	-	-	-	-	20.0	5.21	-	-	-	-
21	<i>Acaulospora scrobiculata</i> Trappe	-	-	-	-	58.3	2.98	-	-	-	-	33.3	34.76	-	-
22	<i>Acaulospora spinosa</i> Walker & Trappe	-	-	14.2	2.47	-	-	-	-	-	-	-	-	-	-
23	<i>Scutellospora gregaria</i> (Schenck & Nicolson) Walker & Sanders	-	-	14.2	3.91	-	-	-	-	-	-	-	-	-	-
24	<i>Scutellospora wershuijzei</i> Koske & Walker	-	-	-	-	-	-	7.1	2.32	-	-	-	-	20.0	1.08
25	<i>Scutellospora dipurpurea</i> Morton & Koske	-	-	14.2	10.10	-	-	-	-	-	-	-	-	-	-
26	<i>Scutellospora calospora</i> (Nicolson & Gerdemann) Walker & Sanders	-	-	-	-	8.3	0.42	-	-	-	-	-	-	-	-
27	<i>Gigaspora albida</i> Schenck & Smith	20.0	2.94	-	-	-	-	14.2	0.35	-	-	16.6	3.07	-	-
28	<i>Entrophospora infrequens</i> (Hall) Ames & Schneider	-	-	-	-	-	-	7.1	0.98	-	-	-	-	-	-

**Table 4. Pearson correlation coefficient ( $r$  value) between spore density (SD) and root colonization (RC), Relative Abundance (RA) and Isolation Frequency (IF), and Spore density and Species richness**

Ecological parameters	Terekhol	Chapora	Mandovi	Zuari	Sal	Talpona	Galgibagh
SD v/s RC	0.670	0.62	0.597	0.376	0.117	0.242	0.552
RA v/s IF	*0.869	*0.894	*0.675	*0.723	*0.718	0.834	*0.817
SD v/s SR	*0.965	0.720	*0.938	*0.925	*0.932	*0.836	*0.937

Notes: \* is significant ( $p \leq 0.05$ ).





**Fig. 2** Arbuscular mycorrhizal fungal species (a–i) & structures (j–o) from mangroves (a) *Acaulospora scrobiculata*, (b) *A. bireticulata*, (c) *A. laevis*, (d) *Glomus rubiforme*, (e) *G. taiwanense*, (f) *G. aggregatum*; (g) *G. geosporum*, (h) *G. hyderabadensis*, (i) characteristic heart-shaped germination shield of *Scutellospora weresubiae*, (j & k) arbuscular colonization in mangrove roots; (l) AMF hyphal coils in mangrove root, (m & n) vesicular colonization in mangrove roots, (o) auxillary cells in *Gigaspora albida*.

## Discussion

Recorded fluctuation in pH and EC levels of mangrove soils might be attributable to the constant flushing of water that leads to deposition of salt (Rodrigues and Anuradha 2009). Padma and Kandaswamy (1990) reported that nearly 80%–85% of P is made unavailable to plants due to fixation and immobilization.

The root hair of the studied mangrove species was small and poorly developed. This feature is known to create potential plant mycotrophy, enhancing nutrient acquisition in stressed environments (Baylis 1975). This study revealed that 94% of mangrove species were mycorrhizal. Similar observations were recorded by Sengupta and Chaudhuri (2002); Kumar and Ghose (2008) and Wang et al. (2010). But this study contradicts the earlier findings of Mohankumar and Mahadevan (1986) who reported absence of AM fungi in mangrove vegetation of the Pitchavaram forest at Tamil Nadu in India. *Paris*-type morphology was present in 74% of the plant species, which is in agreement with the finding of Kubota et al. (2005) who reported dominance of *Paris*-type morphology in natural ecosystems. Brundrett and Kendrick (1990) suggested that slow growth coupled with long root life span and gradual AM fungal colonization exhibited in *Paris*-type morphology may be the best growth strategy under low nutrient and high stress conditions that often prevail in mangrove ecosystems. Bedini et al. (2000) suggested that the genotypic physiology of the host may also contribute to the type of colonization. In *A. ilicifolius*, AM fungal colonization showed insignificant variation by site.

Newsham et al. (1995) suggested overall functionality in AM communities remains fairly constant regardless of species variation. Variation in AM fungal root colonization and spore density was observed. However, no significant correlation was established which is in agreement with Miller (2000). AM fungal colonization is known to depend on soil moisture and P availability (Ruotsalainen et al. 2002; Wang et al. 2010), and physiology, growth rate and turnover of plant roots (Lugo et al. 2003). Similarly, variation in spore density at the seven study sites might be due to environmental fluctuations playing a key role in influencing AM symbiosis. Zhao (1999) reported seasonality, edaphic factors, age of host plants and dormancy might be factors contributing to variations in spore density.

Based on RA and IF, *Glomus* and *Acaulospora* were the dominant genera and *G. intraradices* and *A. laevis* were the dominant species. Bever et al. (1996) reported that *Glomus* and *Acaulospora* species usually produce more spores than *Gigaspora* and *Scutellospora* species within the same environment. Because of their smaller spore size, *Glomus* and *Acaulospora* species require less time to sporulate (Hepper 1984) and are therefore more adaptive in adjustment of sporulation pattern in varied environmental conditions (Stutz and Morton 1996).

Thus, it is important to consider the sporulation characteristics of AM fungi in determining dominance in mangrove communities. Stutz et al. (2000) reported that *Glomus* species are known to be widely distributed and commonly found in different ecosystems and geographical regions. Since the studied mangrove

soils were acidic, this might explain the dominance of *A. laevis*, as reported by Abbott and Robson (1991) for the occurrence of *Acaulospora* species in acidic soils. Climatic and edaphic factors, together with the host species and soil type (Muthukumar and Udaiyan 2002) and differential sporulation ability of AM fungal species (Barni and Siniscalco 2000), might influence AM fungal distribution. The species that produce more spores had wider distribution while species with a smaller geographical range produced fewer spores. Spore production of AM fungi is known to vary greatly by ecosystem type and is affected by many environmental and biological factors (Zhang et al. 2004).

Maximum AM species richness was recorded at Zuari (16) and minimum at Talpona (5) which may be correlated to plant species diversity occurring at the sites. More samples were examined at Zuari because it showed maximum mangrove diversity. Sturmer and Bellei (1994) reported that species richness is dependent on sample number: the more samples collected, the more species are likely to be recovered. Correlation analysis between AM species richness and spore density showed a significant positive correlation at six sites while in Chapora no significant correlation was observed. Ferrol et al. (2004) and Radhika and Rodrigues (2010) reported higher species richness and spore density in terrestrial ecosystems in comparison to the wetland system studied here.

Six of the seven sites showed significant positive correlation between RA and IF. Talpona showed no significant correlation. Some AM species, viz. *G. fasciculatum* at Mandovi and *G. clarum* at Zuari, were not present at high IF but were dominant in sporulation compared to other species. In contrast, *G. mosseae* and *A. scrobiculata* at Mandovi and *G. aggregatum* at Sal had low RA but were widely distributed (high IF). Clapp et al. (1995) suggested that wider distribution and lower RA signifies a strong mycelial network that spreads among host plants over a large area and produces fewer spores. Also, in the present study there was no significant correlation between spore density and root colonization at any site. Shannon Wiener diversity ( $H'$ ) was highest at Talpona and lowest at Zuari. Distribution of AM species was more uniform at Talpona ( $E = 0.41$ ) compared to other sites. Bever et al. (1996) reported that differences in sporulation ability of different AM fungal species can result in unevenness in spore density. Simpson's dominance ( $D$ ) ranged from 0.97 to 0.99.

In this study 28 AM fungal species of five genera were recovered from the rhizosphere soils, more than the six species reported by Wang et al. (2010) for South China. Radhika and Rodrigues (2007) described only four AM fungal species of two genera on aquatic and marshy plant species at Goa, India. Dalpe and Aiken (1998) reported that the persistence of AM fungi in mangroves depends upon survival of propagules like spores, mycelia and colonized root systems, and AM fungal mycelium seem to be morphologically and physiologically well adapted to extreme environments (Klironomos et al. 2001), thus enabling long term survival and sporulation.

In general, the diversity of AM fungi observed in wetland ecosystem is lower than in terrestrial ecosystems (Radhika and Rodrigues 2010; Zhao and Zhao (2007). Spatial and temporal varia-

tions in mangrove ecosystems and the preference of different host plants may be reasons for lower diversity of AM fungi in wetlands (He et al. 2002).

Variation in functional diversity within one AM fungal species can be greater than between different AM fungal species or even genera (Munkvold et al. 2004). This may indicate host preference. Thus, ecological studies are needed to test differential mangrove plant performance in response to AM fungi. Considering their effect on mangrove species, AM fungi may be important drivers of plant community composition in mangrove ecosystems. The fungal species that occurred at all study sites showed different patterns of sporulation and distribution, suggesting differences in functional diversity. Further studies are needed to consider the combined effect of occurrence of AM fungi by season and phenological stage of mangroves.

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#### Appendix 1. Distribution, spore density, root colonization and species richness in mangrove species from selected study sites.

Mangrove plant species		Habit	AMF species	Spore density *	Root colonization (%)	Species Richness
Site, family	Species					
<b>I- Terekhol</b>			3, 1, 12, 14, 15, 19, 27	-	-	-
Acanthaceae	<i>Avicennia marina</i> (Forsk.) Vierh	TM	1	0.27	6	1
	<i>Acanthus ilicifolius</i> L.	TM	3, 27, 14	1.48	60	3
Rhizophoraceae	<i>Rhizophora apiculata</i> Blume.	TM	1, 15	0.42	13	2
	<i>Ceriops tagal</i> (Perr.) C.B. Robinson	TM	1, 3, 12, 19	1.92	32	4
Sonneratiaceae	<i>Sonneratia caseolaris</i> (L.) Engler	TM	14	0.32	46	1
Average data				0.88	31.4	
<b>II- Chapora</b>			1, 6, 19, 22, 23, 25			
Rhizophoraceae	<i>Rhizophora mucronata</i> Poir.	TM	1, 25	0.4	12	2
Acanthaceae	<i>Acanthus ilicifolius</i> L.	TM	1, 19, 25	0.49	42	3
	<i>Avicennia officinalis</i> L	TM	6, 19	1.72	21	2
	<i>Avicennia marina</i> (Forsk.) Vierh	TM	25	0.19	6	1
Euphorbiaceae	<i>Excoecaria agallocha</i> L.	TM	1, 6, 22, 23	2.4	58	4
Myrsinaceae	<i>Aegiceras corniculatum</i> (L) Blanco	TM	19	0.34	12	1
Salvadoraceae	<i>Salvadora persica</i> L.	MA	-	-	-	-
Average data				0.99	25.1	



## Continued Appendix 1

Mangrove plant species		Habit	AMF species	Spore density *	Root colonization (%)	Species Richness
Site, family	Species					
Site, family	Species					
Sonneratiaceae	<i>Sonneratia alba</i> (L.) Smith.	TM	5	0.13	23	1
	<i>Sonneratia caseolaris</i> (L.) Engler	TM	1, 5, 17, 21	1.32	38	4
Acanthaceae	<i>Acanthus ilicifolius</i> L.	TM	1, 5, 14, 19, 21	2.45	69	5
	<i>Avicennia officinalis</i> L.	TM	14	0.14	12	1
Ceratopteridaceae	<i>Acrostichum aureum</i> L.	MA	17, 19	0.57	72	2
Fabaceae	<i>Derris heterophylla</i> Willd.	MA	13	0.08	14	1
Rhizophoraceae	<i>Kandelia candel</i> (L.) Druce.	TM	4	0.17	15	1
	<i>Rhizophora apiculata</i> Blume.	TM	13, 14, 19, 21	1.87	28	4
	<i>Rhizophora mucronata</i> Poir.	TM	1, 26	0.58	41	2
Euphorbiaceae	<i>Excoecaria agallocha</i> L.	TM	1, 4, 13, 18	1.76	77	4
Salvadoraceae	<i>Salvadora persica</i> L.	MA	-	-	-	-
Myrsinaceae	<i>Aegiceras corniculatum</i> (L.) Blanco	TM	14, 21	0.42	42	2
Average data				0.86	35.3	
IV- Zuari						
				1, 2, 3, 5, 7, 8, 10, 11, 12, 13, 16, 17, 19, 24, 27, 28		
Ceratopteridaceae	<i>Acrostichum aureum</i> L.	MA	2, 17, 19	0.89	17	3
Fabaceae	<i>Derris heterophylla</i> Willd.	MA	12	0.13	7	1
Rhizophoraceae	<i>Bruguiera cylindrica</i> (L.) Bl.	TM	1, 8, 10, 11, 13	2.34	9	5
	<i>Bruguiera gymnorrhiza</i> (L.) Lam.	TM	8	0.24	19	1
	<i>Rhizophora apiculata</i> Blume.	TM	1, 27	1.36	19	2
Acanthaceae	<i>Rhizophora mucronata</i> Poir.	TM	1, 19, 27	0.76	26	3
	<i>Acanthus ilicifolius</i> L.	TM	1, 7, 13, 16, 19, 24, 28	2.84	53	7
	<i>Avicennia marina</i> (Forsk.) Vierh	TM	-	-	18	-
Salvadoraceae	<i>Salvadora persica</i> L.	MA	-	-	-	-
Euphorbiaceae	<i>Excoecaria agallocha</i> L.	TM	1, 3, 7, 11	0.98	42	4
Sonneratiaceae	<i>Sonneratia caseolaris</i> (L.) Engler	TM	7, 13	0.33	27	2
	<i>Sonneratia alba</i> (L.) Smith.	TM	-	-	29	-
Average data				1.22	23.6	
V-Sal						
				1, 4, 7, 13, 14, 19, 20, 26		
Acanthaceae	<i>Acanthus ilicifolius</i> L.	TM	1, 13, 19, 26	3.24	36	4
	<i>Avicennia officinalis</i> L.	TM	7, 14, 19	1.8	7	3
Rhizophoraceae	<i>Rhizophora mucronata</i> Poir.	TM	4, 19, 20	1.45	28	3
Sonneratiaceae	<i>Sonneratia alba</i> (L.) Smith.	TM	1, 19,	0.86	40	2
Average data				1.84	27.7	
VI- Talpona						
				1, 2, 19, 21, 27		
Acanthaceae	<i>Acanthus ilicifolius</i> L.	TM	1, 21	0.53	65	2
	<i>Avicennia officinalis</i> L.	TM	19	0.14	19	1
	<i>Avicennia alba</i> Blume.	TM	2	0.11	48	1
Rhizophoraceae	<i>Bruguiera cylindrica</i> (L.) Bl.	TM	2, 19, 27	1.72	14	3
	<i>Bruguiera gymnorrhiza</i> (L.) Lam.	TM	21	0.1	17	1
	<i>Rhizophora mucronata</i> Poir.	TM	1, 2, 19	0.65	42	3
Average data				0.54	34.1	
VII- Galgibagh						
				1, 2, 6, 9, 19, 24		
Euphorbiaceae	<i>Excoecaria agallocha</i> L.	TM	1, 2, 19	1.64	34	3
Acanthaceae	<i>Acanthus ilicifolius</i> L.	TM	2, 19, 24	2.28	64	3
	<i>Avicennia officinalis</i> L.	TM	9	0.17	27	1
Rhizophoraceae	<i>Bruguiera cylindrica</i> (L.) Bl.	TM	1, 6	0.49	60	2
	<i>Rhizophora apiculata</i> Blume.	TM	19	0.12	29	1
Average data				0.94	42.8	

**Notes:** Total Soil samples is 51, average spore density is  $1.03 \pm 0.04$ ; Average species richness is  $8.5 \pm 3.9$ . TM is True mangrove; MA is Mangrove associate; \* is spores- $g^{-1}$  of soil; Number listed in column labelled AM fungal species correspond to Table 3.